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# HYPOVOLEMIC HEMORRHAGE INDUCES FOS EXPRESSION IN THE RAT HYPOTHALAMUS: EVIDENCE FOR INVOLVEMENT OF THE LATERAL HYPOTHALAMUS IN THE DECOMPENSATORY PHASE OF HEMORRHAGE

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**Abstract—This study tested the hypothesis that the hypothalamus participates in the decompensatory phase of hemorrhage by measuring Fos immunoreactivity and by inhibiting neuronal activity in selected hypothalamic nuclei with lidocaine or cobalt chloride. Previously, we reported that inactivation of the arcuate nucleus inhibited, but did not fully prevent, the fall in arterial pressure evoked by hypotensive hemorrhage. Here, we report that hemorrhage (2.2 ml/100 g body weight over 20 min) induced Fos expression in a high percentage of cells in the paraventricular, supraoptic and arcuate nuclei of the hypothalamus as shown previously. Lower densities of Fos immunoreactive cells were also found in the medial preoptic area (mPOA), anterior hypothalamus, lateral hypothalamus (LH), dorsomedial hypothalamus, ventromedial hypothalamus (VMH) and posterior hypothalamus. Bilateral injection of lidocaine (2%; 0.1 μl or 0.3 μl) or cobalt chloride (5 mM; 0.3 μl) into the tuberal portion of the LH immediately before hemorrhage was initiated reduced the magnitude of hemorrhagic hypotension and bradycardia significantly. Lidocaine injection into the VMH also attenuated the fall in arterial pressure and heart rate evoked by hemorrhage although inactivation of the mPOA or rostral LH was ineffective. These findings indicate that hemorrhage activates neurons throughout much of the hypothalamus and that a relatively broad area of the hypothalamus, extending from the arcuate nucleus laterally through the caudal VMH and tuberal LH, plays an important role in the decompensatory phase of hemorrhage.**


**Key words: hemorrhage, hemorrhagic shock, lateral hypothalamus, ventromedial hypothalamus, arcuate nucleus, Fos immunoreactivity.**

## INTRODUCTION

Severe hemorrhage produces a biphasic, centrally mediated cardiovascular response. Initially, arterial pressure is maintained within normal limits through a compensatory, baroreceptor-mediated increase in sympathetic drive (Schadt and Ludbrook, 1991; Evans et al., 2001). But if blood loss continues beyond 25–30% of total blood volume a second, decompensatory phase ensues in which sympathetic activity decreases abruptly and arterial pressure and heart rate fall. The decompensatory phase of hemorrhage is thought to be triggered by cardiac mechanoreceptors that convey information about blood volume to the brain through vagus nerve afferents (Oberg and Thorén, 1972; Schadt and Ludbrook, 1991) although recent studies suggest that cardiac spinal nerves may be principally involved (Troy et al., 2014). Sympathetic outflow is inhibited through a sequential, descending neuronal pathway extending from the ventrolateral column of the midbrain periaqueductal gray region (vlPAG) to the midline raphe nuclei and rostral ventrolateral medulla (Carrive and Bandler, 1991; Lovick, 1993; Coleman and Dampney, 1995; Henderson et al., 1998a; Dean, 2004; Vagg et al., 2008). The anatomical pathway that conveys the signal produced by hypovolemia from cardiac nerves to the vlPAG has not been fully elaborated, however.

In an earlier study, we tested the hypothesis that the arcuate nucleus of the hypothalamus participates in the response to hemorrhage (Göktalay et al., 2006). We found that hypotensive, but not non-hypotensive, hemorrhage induced robust expression of the immediate/early gene product Fos in the arcuate nucleus, suggesting that hemorrhage activates arcuate neurons (Göktalay et al., 2006). Conversely, bilateral injection of the local anesthetic lidocaine into the arcuate nucleus to inhibit neuronal activation attenuated, although it did not completely

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*Abbreviations:* AH, anterior hypothalamus; ANOVA, analysis of variance; Arc, arcuate nucleus; BST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamus; f, fornix; LH, lateral hypothalamus; LPO, lateral preoptic area; MAP, mean arterial pressure; MnPO, median preoptic nucleus; MPO, medial preoptic nucleus; mPOA, medial preoptic area; mt, mammillothalamic tract; opt, optic tract; OX, optic chiasm; PAG, periaqueductal gray region; PBS, phosphate-buffered saline; PH, posterior hypothalamus; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; SON, supraoptic nucleus; VLH, ventrolateral nucleus; vlPAG, ventrolateral periaqueductal gray region; VMH, ventromedial nucleus; 3V, third ventricle.

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abolish, the fall in arterial pressure evoked by hemorrhage (Göktalay et al., 2006). These findings suggest that the arcuate nucleus plays an important role in the decompensatory phase of hemorrhage but the incomplete inhibition of the response raises the possibility that adjacent regions of the hypothalamus may also be involved.

The hypothesis that the arcuate nucleus and, perhaps, adjacent hypothalamic nuclei mediate the decompensatory phase hemorrhage was predicated on extensive evidence that the hypothalamus influences cardiovascular function. Physiologic studies, some dating back 80 years or more, have repeatedly demonstrated that electrical stimulation of, or excitatory amino acid injection into, the hypothalamus either increases or decreases arterial pressure and heart rate depending on the location (Ranson et al., 1935; Hilton, 1982; Gelsema et al., 1989; DiMicco et al., 2002; Sévoz-Couche et al., 2006; Deolindo et al., 2008). Pressor sites are found primarily in the paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), portions of the anterior hypothalamus (AH) and medial aspects of the lateral hypothalamus (LH) (Gelsema et al., 1989; Allen and Cechetto, 1992). Depressor sites are distributed throughout the rostro-caudal extent of the hypothalamus, primarily in its lateral aspects, with little correspondence to traditional boundaries of hypothalamic nuclei (Hilton, 1982; Gelsema et al., 1989; Allen and Cechetto, 1992; Göktalay et al., 2004; Pajolla and Corrêa, 2004). The functional significance of these cardiovascular responses has not been thoroughly evaluated and it is not known whether hypothalamic subregions other than the arcuate nucleus participate in the physiologic response to hemorrhage.

To further investigate the hypothalamic areas that participate in the effects of hemorrhage we conducted two series of experiments. First, we measured Fos immunoreactivity to map the distribution of hypothalamic neurons activated by hemorrhage. A number of previous studies have used Fos expression to study the effects of hemorrhage on the PVN and supraoptic nucleus (SON) (Badoer et al., 1993; Buller et al., 1999; Jaworski et al., 2002) although, surprisingly, the effect of hemorrhage on Fos expression in other hypothalamic subregions has not been comprehensively investigated. Secondly, we investigated whether hypothalamic areas in close proximity to the arcuate nucleus participate in the decompensatory phase of hemorrhage by testing whether lidocaine or cobalt chloride injection into specific hypothalamic nuclei prevents the fall in arterial pressure and heart rate evoked by acute hemorrhage. Here we report evidence that hemorrhagic hypotension is mediated by a relatively broad region of the hypothalamus extending laterally from the arcuate nucleus to the caudal ventromedial hypothalamus (VMH) and tuberal LH.

## EXPERIMENTAL PROCEDURES

### Animals

Male Sprague–Dawley rats (250–300 g; Charles River Laboratories, Wilmington, MA, USA) were housed in groups of three in a temperature-controlled room with free access to food and water and ambient lighting between 7:00 a.m. and 7:00 p.m. The animals were allowed to acclimate to the animal facility for at least five days and were handled daily for three days before each experiment. The experiments were conducted between 9:00 a.m. and 12:00 p.m. The animal protocols were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

### Hemorrhage and tissue processing

Rats were anesthetized with 4% halothane (Sigma–Aldrich, Inc., St. Louis, MO, USA) and maintained under anesthesia with 1.5% halothane in 100% $O_2$. The left femoral artery of each rat was cannulated with PE 50 tubing filled with sterile heparinized glycerol (250 U/ml) under sterile surgical conditions. The cannula was exteriorized at the nape of the neck, the wound was sutured closed and the animal was returned to its home cage after recovering from anesthesia. The animals were allowed to recover from surgery for five days and were handled daily.

Arterial pressure was measured in unrestrained animals that were allowed to remain in their home cages to control for effects of stress. At the beginning of each experiment the arterial cannula was attached to a low volume pressure transducer (TXD-300; MicroMed, Inc., Louisville, KY, USA) connected to a MicroMed BPA-200 digital pressure monitor (Model BPA-200 Blood Pressure Analyzer; MicroMed, Inc., Louisville, KY, USA) and diastolic, systolic and mean arterial pressure and heart rate were recorded at one-min intervals for 60 min. Data were analyzed and stored using a MicroMed data acquisition system (System Integrator; MicroMed, Inc.).

Hemorrhage was initiated by disconnecting the arterial cannula from the pressure transducer and allowing blood (2.2 ml/100 g body wt) to flow through the femoral arterial catheter at a controlled rate for 20 min. Arterial pressure was maintained at approximately 40 mmHg; if necessary, additional blood was removed. One hour after completion of the hemorrhage procedure rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, ip; Sigma–Aldrich, Inc., St. Louis, MO, USA) and perfused transcardially with 100 ml 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 300 ml PBS containing 3% paraformaldehyde and 2.5% sucrose. Brains were removed and post-fixed in 3% paraformaldehyde for 2 h at 4 °C, rinsed three times with PBS then transferred to 10% sucrose for 1 h and stored in 20% sucrose overnight at 4 °C. Sham-operated control animals were treated in the same way except that blood was not withdrawn. A second control group of rats were anesthetized with sodium pentobarbital in their home cages and sacrificed immediately without undergoing surgery or other manipulations.

### Fos immunohistochemistry

Fos immunohistochemistry was performed using a rabbit polyclonal antiserum raised against a synthetic peptide